Amendment to the Claims

1-18. (canceled)

19. (previously presented) A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen;

adding to said sample an inhibitor of cytokine secretion;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

20. (previously presented) The method of claim 19, further comprising the step of adding to said sample, contemporaneously with antigen contact, a costimulus of T cell activation.

21. (previously presented) The method of claim 20, wherein said costimulus is an antibody specific for CD28.

22. (previously presented) The method of claim 20, wherein said costimulus is an antibody specific for VLA-4.

23. (previously presented) The method of claim 19, further comprising contacting said sample with an antibody specific for a T lymphocyte early activation antigen, and then flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset that concurrently bind said early activation antigen-specific antibody.

24. (previously presented) The method of claim 23, wherein said T lymphocyte early activation antigen is CD69.

25. (canceled).

26. (previously presented) The method of any one of claims 19, 20, 23, or 25 wherein said sample is a whole blood sample.

27. (previously presented) The method of claim 26, further comprising the step of adding a cationic chelator after antigen contact is complete but prior to flow cytometric detection.

28. (previously presented) The method of claim 27, wherein said chelator is EDTA or EGTA.

29. (previously presented) The method of claim 28, wherein said chelator is EDTA.

30. (previously presented) The method of claim 26, further comprising the step of lysing red blood cells.

31. (previously presented) The method of claim 19, wherein said nominal antigen is selected from the group consisting of alloantigens, autoantigens, viral antigens, and bacterial antigens.

32. (previously presented) The method of claim 31, wherein said nominal antigen is a viral antigen.

33. (previously presented) The method of claim 32, wherein said antigen is a CMV antigen.

34. (canceled).

35. (previously presented) The method of claim 32, wherein said antigen is a mumps antigen.

36. (previously presented) The method of claim 32, wherein said antigen is a measles antigen.

37. (previously presented) The method of claim 31, wherein said MHC-dependent nominal antigen is a bacterial antigen.

38. (previously presented) The method of claim 37, wherein said antigen is a Mycobacterium tuberculosis antigen.

39. (previously presented) The method of claim 19, wherein said inhibitor of cytokine secretion is Brefeldin A.

40. (previously presented) The method of claim 19, wherein said cytokine-specific antibody is specific for a cytokine selected from the group consisting of: IL-2, IL-4, IL-13, γ-IFN, and TNF-α.

41. (previously presented) The method of claim 40, wherein said cytokine-specific antibody is specific for IL-2.

42. (previously presented) The method of claim 40, wherein said cytokine-specific antibody is specific for IL-4.

43. (previously presented) The method of claim 40, wherein said cytokine-specific antibody is specific for γ-IFN.

44. (previously presented) The method of claim 40, wherein said cytokine-specific antibody is specific for TNF-α.

45. (previously presented) The method of claim 19, wherein said T lymphocyte subset-defining antibody is selected from the group consisting of antibodies specific for: CD3, CD4, CD8, TCR, homing receptors, CD45RO, CD45RA and CD27.

46. (previously presented) The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD3.

47. (previously presented) The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD4.

48. (previously presented) The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD8.

49. (previously presented) The method of any one of claims 19, 20, or 23 wherein said anti-cytokine antibodies, said T lymphocyte subset-defining antibodies, and said early activation antigen-specific antibodies are each conjugated directly to fluorophores.

50. (previously presented) The method of claim 49, wherein said fluorophores are selected from the group consisting of FITC, PE, PerCP, and APC.

51. (previously presented) The method of claim 50, wherein said anti-cytokine antibodies are conjugated to FITC.

52. (previously presented) The method of claim 50, wherein said T lymphocyte subset-defining antibodies are conjugated to PerCP.

53. (previously presented) The method of claim 50, wherein said antibody specific for a T lymphocyte early activation antigen is conjugated to PE.

54. (previously presented) The method of any one of claims 19, 20, or 23 wherein said antigen-contacting step lasts no longer than 24 hours.

55. (previously presented) The method of claim 54, wherein said antigen-contacting step lasts no longer than 6 hours.

56 (canceled).

57 (canceled).

58 (canceled).

59 (canceled).

60 (canceled).

61. (previously presented) The method of claim 19, wherein each of said at least one cytokine-specific antibody is specific for a cytokine selected from the group consisting of IL-2, IL-4, IL-13, IFN-γ, and TNF-α.

62. (previously presented) The method of claim 61, further comprising the step of adding to said sample, contemporaneously with antigen contact, a costimulus of T cell activation, wherein said costimulus is selected from the group consisting of antibodies specific for CD28, VLA-4, CD86, or CD118.

63. (previously presented) The method of claim 61, further comprising contacting said sample with an antibody specific for CD69, and then flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by CD69+ cells in the defined T lymphocyte subset.

64. (previously presented) A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

65. (previously presented) A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A, wherein said culturing is carried out in a slant tube;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

66. (currently amended) ~~The method of claim 65~~ A method of detecting T lymphocytes that are specific for a nominal antigen, comprising:

culturing a sample containing peripheral blood mononuclear cells with a nominal antigen in the presence of Brefeldin-A, wherein said culturing is carried out in a slant tube;

permeabilizing said cells;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset, wherein said step of flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset comprises analyzing at least 50,000 cells.